



## APPENDIX B

1. A vaccine composition comprising an immunologically protective amount of a first attenuated, non-reverting mutant *Salmonella* bacterium in which two or more genes within the SPI2 region have been inactivated.

2. The vaccine composition of claim 1 wherein said genes are inactivated by deletion of a portion of the coding region of the gene.

3. The vaccine composition of claim 1 wherein said genes are inactivated by an insertional mutation.

4. The vaccine composition of claim 1 wherein the genes are (*ssa*) secretion system apparatus genes.

5. The vaccine composition of claim 1 wherein the genes are selected from the group consisting of *ssaT*, *ssaJ*, *ssaC* and *ssaM*, and wherein:

(a) said *ssaT* gene consists of SEQ ID NO: 1 or 2, or a full length nucleotide sequence that hybridizes to the non coding complement of SEQ ID NO: 1 or 2 under stringent conditions, or a full length Ssa-encoding nucleotide sequence that has 95% sequence identity to SEQ ID NO: 1 or 2;

(b) said *ssaJ* gene consists of SEQ ID NO: 3 or 4, or a full length nucleotide sequence that hybridizes to the non coding complement of SEQ ID NO: 3 or 4 under stringent conditions, or a full length Ssa-encoding nucleotide sequence that has 95% sequence identity to SEQ ID NO: 3 or 4;

(c) said *ssaC* gene consists of SEQ ID NO: 5 or 6, or a full length nucleotide sequence that hybridizes to the non coding complement of SEQ ID NO: 5 or 6 under stringent conditions, or a full length Ssa-encoding nucleotide sequence that has 95% sequence identity to SEQ ID NO: 5 or 6; and

(d) said *ssaM* gene consists of SEQ ID NO: 7 or 30, or a full length nucleotide sequence that hybridizes to the non coding complement of SEQ ID NO: 7 or 30 under stringent conditions, or a full length Ssa-encoding nucleotide sequence that has 95% sequence identity to SEQ ID NO: 7 or 30;

and further wherein stringent hybridization conditions comprise hybridization in 50% formamide with washing at 65°C.

6. The vaccine composition of claim 5 wherein, in said first attenuated mutant *Salmonella* bacterium, *ssaT* and *ssaC* have been inactivated.
7. The vaccine composition of claim 2 wherein, in said first attenuated mutant *Salmonella* bacterium, *ssaT* and *ssaJ* have been inactivated.
8. The vaccine composition of claim 1 further comprising a second attenuated mutant *Salmonella* bacterium in which one or more genes within the SPI2 region have been inactivated.
9. The vaccine composition of claim 8 wherein said genes are selected from the group consisting of *ssaT*, *ssaJ*, *ssaC*, and *ssaM*.
10. The vaccine composition of claim 8 wherein said first and second mutant *Salmonella* bacteria are from different serogroups.
11. The vaccine composition of claim 1 or 8 wherein said *Salmonella* bacteria are *Salmonella enterica* subsp *Enterica*.
12. The vaccine composition of claim 1 or 8 wherein said *Salmonella* bacteria are from any of serogroups A, B, C<sub>1</sub>, C<sub>2</sub>, D<sub>1</sub> or E<sub>1</sub>.
13. The vaccine composition of claim 8 wherein said first and second attenuated mutant *Salmonella* bacteria are selected from the group consisting of *S. dublin* and *S. typhimurium*.
14. The vaccine composition of claim 1 wherein said first attenuated mutant *Salmonella* bacterium further comprises a polynucleotide encoding a non-*Salmonella* polypeptide.